1933

A Comparative Study of the Toxic Effects of Colloidal Arsenic and Certain Inorganic Arsenicals on the Grasshopper (Helanopus Differentialis)

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A COMPARATIVE STUDY OF THE TOXIC EFFECTS OF COLLOIDAL ARSENIC AND CERTAIN INORGANIC ARSENICALS ON THE GRASSHOPPER (MELANOPUS DIFFERENTIALIS)

By

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INTRODUCTION

During the spring and summer of 1931 there occurred in south-central South Dakota and northeastern Nebraska the most serious grasshopper outbreak recorded in the United States since the days of the Rocky Mountain locust. The almost complete destruction of all cultivated crops over an area of thousands of square miles in extent, ranks this outbreak as one of the most serious insect plagues which has ever confronted the farmers of the West.

According to Parker & Shotwell (1931) grasshoppers (Melanopus bivattatus and Melanopus differentialis) destroyed 75 per cent of the crops over an area 17,000 square miles in extent and 25 per cent of the crops over an additional 13,000 square miles.

According to A. L. Ford, Extension Entomologist of South Dakota State College, "grasshoppers are a menace to crops in one or more parts of South Dakota almost every year. However, the outbreak in 1931 was the most severe in the history of the state. In spite of the fact that at least 7,493 tons of poisoned bran mash were used in South Dakota in 1931 and by far the most of it in three counties, the crops in the heavily infested areas were 100 per cent destroyed. Grasshoppers totally destroyed the crop over an area of approximately 300 townships or nearly 11,000 square miles. This tremendous damage was helped along by the most severe drought experienced in the history of the state."

A. L. Ford further states, "the infestation in Lyman, Tripp, Gregory, Jones, Mellette, Stanley, Hughes, Sully, Buffalo, Brule, and parts of Charles Mix counties was the heaviest the specialist has ever seen. In certain localities in this area, we estimated as high as 20,000 eggs per square foot of buffalo sod, all eggs being hatchable and in good condition. During the height of the outbreak in heavily infested fields, fully 500 adult hoppers were present per square yard. Under such unusual infestation, the poison failed to save crops even though the normal 70 or 80 per cent kill was obtained. It is still questionable in the mind of the specialist whether or not poisoned bait will satisfactorily control grasshoppers under such terrific infestations."

In regard to outlook for 1933 Ford states "although indications show that there will be heavy grasshopper infestations in localized areas in the central part of the state, the situation is less serious than for 1931 and 1932. Heavy infestations cover about one-third as much area in the state as such infestations covered the previous year (1932)."

Poison bran baits have been used for the control of locusts since they were first introduced into California in 1885. However, there are many criticisms of the poison bran material. It is slow in action, toxic to higher animals and it is not known to be particularly attractive. The recent outbreak brought these questions to the fore and the problem of more effective control presented itself.

Any student of insecticides cannot help but be impressed by the fact that slight changes in the physical factors of an insecticide may cause a great difference in the effectiveness of the compound. An emulsion with large particles behaves differently from one with small particles. A metallic substance such as arsenate of lead, precipitated in the presence of a colloid, is far more finely divided and far superior in adhesive and suspensory values than the ordinary precipitated form.

Wardle (26) states that from the wide range of substances whose toxicity to insects would seem to recommend their use as insecticides for chewing insects, there is as yet no substance or group of substances whose use would seem to threaten the widespread popularity of arsenical compounds for this purpose. Accordingly our search for some more effective insecticide was directed along the line of slightly changing the physical form of some arsenical already known. The question of toxicity to higher animals was also one which had to be seriously considered especially since there were many cases of stock being poisoned from baits commonly used at present.

The success of Erlich in developing the compound Salvarsan which is highly toxic to protozoan parasites and yet relatively non-toxic to humans suggested that some colloidal form of arsenic (as Salvarsan is colloidal in form) might be quite successful in the control of insects. Such then is the method by which we arrived at the study of colloidal arsenious sulphide as an insecticide.

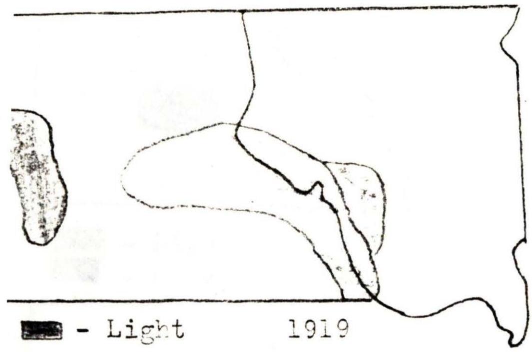
The question of arsenical residue is one which has come to the fore of late years in view of the appreciable quantities of arsenic which have been recovered from the outer surfaces of fruits which have been subjected to late spraying. According to the findings of the Royal Commission on arsenical poisoning of 1903, foodstuffs used for human consumption must not contain more than 0.01 grain of arsenic trioxide equivalent per pound (1.429 milligrams per kilogram). During recent years, public analysts in Great Britain have brought forward examples of apples - in every case imported from areas where late spraying with adhesive arsenicals is carried out - where the amount of arsenic adherent to the apple surface was in excess of the British limit; and in view of the tendency of Public Health officials to condemn any consignment of apples in which samples show a greater arsenic content than 0.01 grain per pound, the matter is of considerable importance to the American and Australian grower, since it may involve a change of spray procedure. A colloid being relatively unstable and comparatively easy to precipitate should be quite adaptable in this problem.

The arsenical residue situation has also emphasized the desirability of comparing the toxicity of stomach poisons for mammals and insects. A stomach-poison, ideal in this respect, would be highly toxic to insects and non-toxic to mammals. A measure of the factor of safety should be determined. The factor of safety will be the ratio of the Minimum Lethal Dose for the mammal to that for the insect. The literature regarding M. L. D. for mammals is considerably more extensive than that concerning insects.

In view of the fact that laboratory specimens of grasshoppers (Melanopus differentialis) were obtainable it was deemed important to determine the minimum lethal dose for the grasshopper with colloidal arsenic as compared to other compounds containing arsenic as the chief toxic substance.

GRASSHOPPERS

Grasshoppers are a menace to crops in one or more parts of South Dakota almost every year. The accompanying maps show the location and extent of grasshopper infestation in the state for the past 14 years. #



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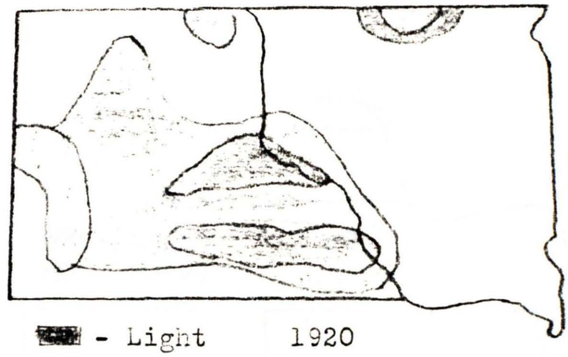

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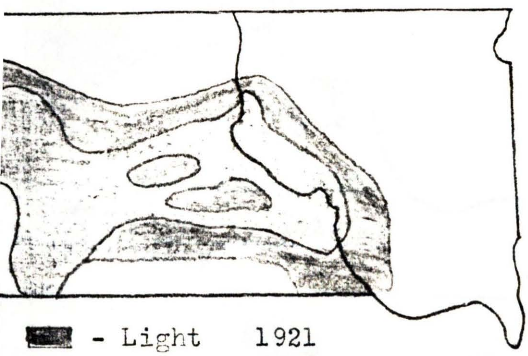

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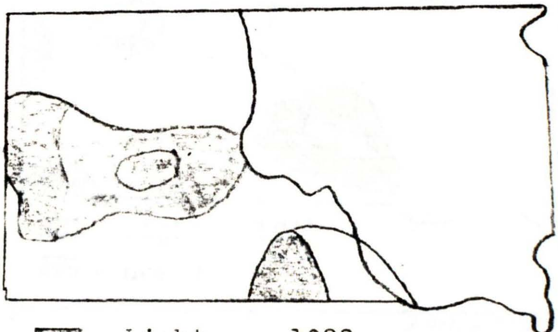

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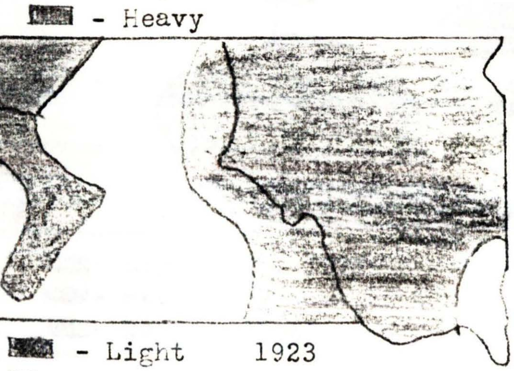

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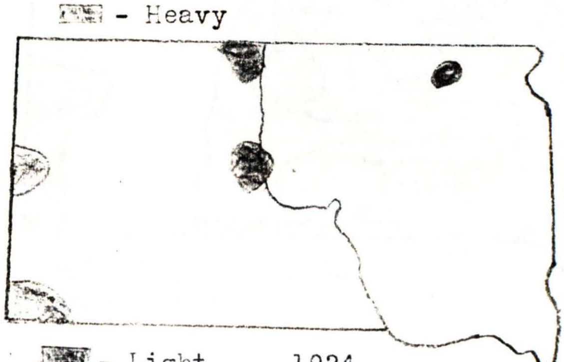

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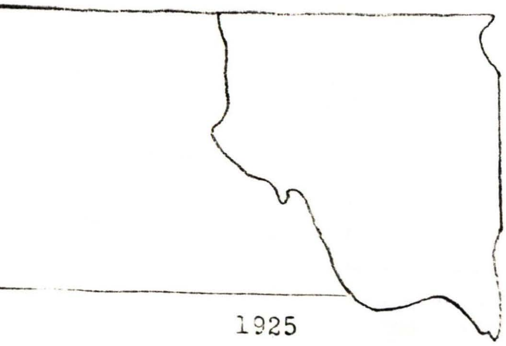

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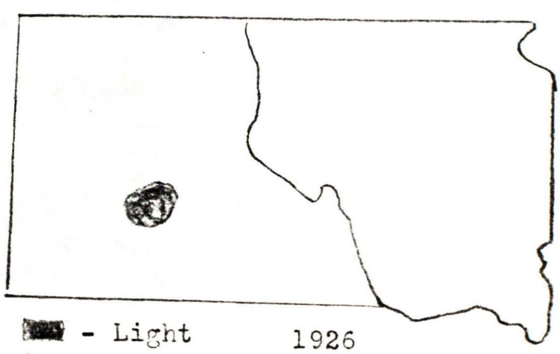

- Light 1926
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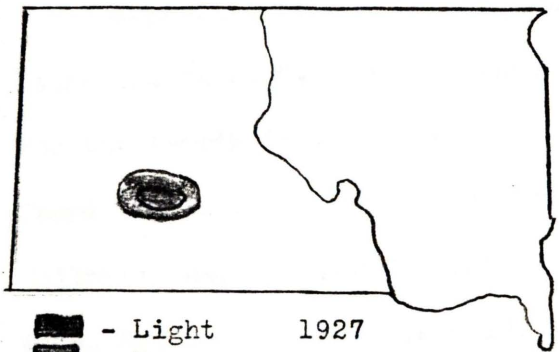

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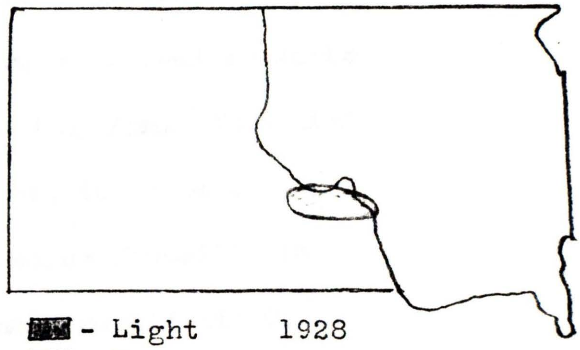

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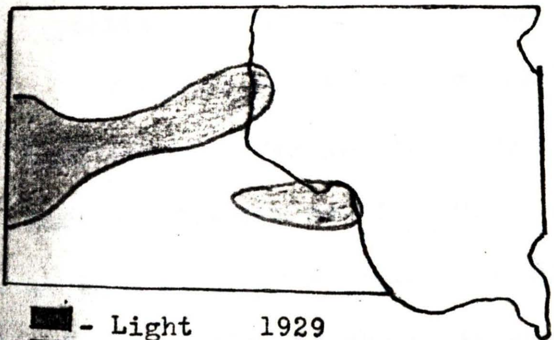

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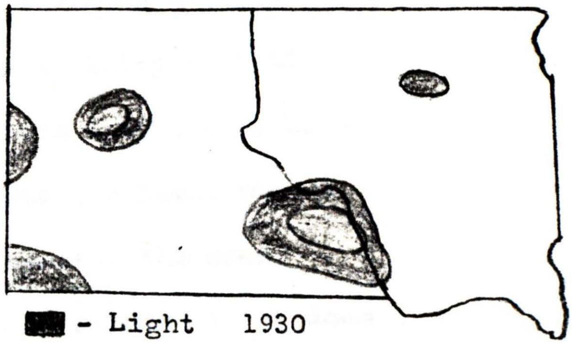

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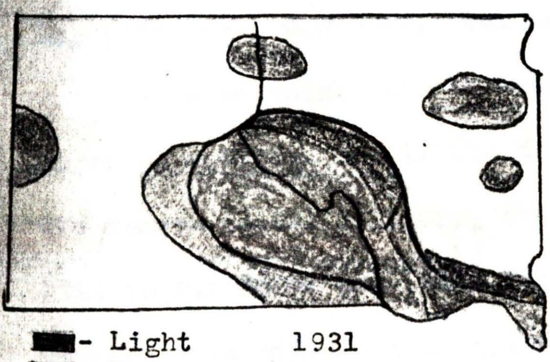
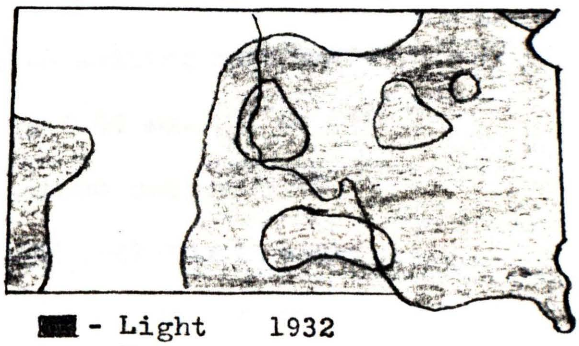


- Light 1931
- Heavy
- Devastation



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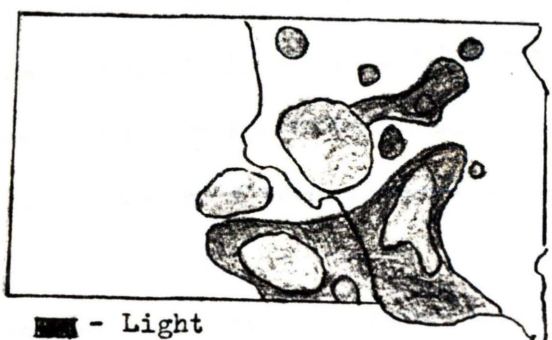

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Outlook for 1933

These maps were prepared from copies furnished by A. L. Ford, Extension Specialist.

REVIEW OF LITERATURE ON TOXICITY.

Campbell & Filmer (8) by means of the sandwich method found the M. L. D. (Median lethal dose) of acid lead arsenate for the fourth instar silk worm to be 0.09 mg./gm. They also found the M. L. D. for several other compounds to be as follows: copper cyanide 0.04 mg./gm., Sodium fluosilicate 0.09, Barium fluosilicate 0.13, Potassium fluosilicate 0.14, Cryolite 0.18, Basic lead arsenate 0.9, Aluminum arsenate 0.9.

The work of Van Leeuwen (25) on the toxicity of acid lead arsenate for the Japanese beetle shows that the M. L. D. for the silkworm could be used to estimate the least amount of this compound necessary to kill the beetle, the mean weight of which is about .10 gram. The calculated least dose is, therefore, 0.009 mg. Van Leeuwen found it to be 0.0035 gm.

The mean weight of the newly hatched codling moth larva is about 0.04 mg. and if the M. L. D. is 0.09 mg./gm. as for the fourth instar silkworm, the least amount necessary to kill is 0.0000036 gm. This figure should probably be lower because susceptibility to arsenic decreases with the increasing age of larvae.

The fact that a basic lead arsenate may be only 1/10 as toxic as acid lead arsenate is of some interest, because basic compounds have long been known to be much less effective than acid lead arsenate and have been dropped from commercial use.

Richardson and Haas (20) recently found the median lethal dose of acid lead arsenate for potato beetle (Leptinotarsa decemlineata Say) larvae to be 0.30 mg. per gram, or about three times that for the silkworm.

Bulger (4) states that Campbell also has some unpublished results which indicate that the median lethal dose of acid lead arsenate for the adult differential grasshopper (Melanopus differentialis) may be ten times or more as great as that for the silkworm.

Richardson and Haas (19) (1932) have done the only work recorded so far regarding the toxicity of various compounds to grasshoppers. The estimated median lethal dose (M. L. D.) of arsenious oxide is 0.36 milligram per gram of body weight. For the sodium arsenites, Paris green and sodium fluosilicate, the M. L. D.'s are approximately equal and estimated to be about 0.16 mg./gm. The M. L. D. for acid lead arsenate is 3.0 mg./gm. and for rotenone probably 2.0 mg./gm. For survival periods less than 96 hours, monosodium arsenate was more toxic than other compounds.

Campbell and Lukens (9) by a radioactive indicator method found that at least 25 per cent of a moderate lethal dose of acid lead arsenate goes into solution within the gut of a silkworm during its survival period. Acid lead arsenate was found to be much more soluble than basic lead arsenate within the gut.

They also discovered that about half of the dose of lead was voided during the second hour. The rate of evacuation of lead then decreased gradually and at the end of 5 hours 80 per cent of the lead had passed out of the insect. At the end of 24 hours 97.6 per cent of the dose of lead had been voided by the insect, only 2.4 per cent remaining in the larva when it was washed. This does not necessarily mean that 97.6 per cent of the dose of acid lead arsenate was voided in 24 hours because arsenic may have been voided more or less rapidly than lead following the decomposition of part of the acid lead arsenate in the gut.

Haseman (14) found the lethal dose of lead arsenate to be (.0005) grams for the older larvae of apple worms.

Richardson & Thurber (21) (1932) estimated the M. L. D. for the grasshopper for three compounds; arsenious oxide, sodium fluoride, and cuprous cyanide to be an average of 0.11 milligram per gram of body weight. For zinc phosphide it is 0.52 mg./gm. and for acid lead arsenate from 2 to 4 mg./gm.

McIndoo & Cook (17) found 0.0273 milligram of metallic arsenic as the fatal dosage for fully grown silkworms. They also state that an analysis of 59 dead silkworms, each of which had eaten three drops of the arsenical, gave 0.0027 milligram of arsenic per larva indicating that 90 per cent of the arsenic eaten had been voided in the feces before the larva died. The poison used in this case was acid lead arsenate.

TOXIC EFFECTS ON ANIMALS

Farm animals, especially cattle, are attracted by the brackish sweet taste of arsenic solutions, according to Bevan (2). He reports that in 1908 many cattle were accidentally poisoned in the attempts to exterminate the locust with sodium arsenite.

Since arsenic is attractive to animals, they may easily secure a fatal dose from treated plants or from poison bait or even from the ground where arsenic has been applied. Blyth (3) sets the standard medicinal dose of arsenic (As_2O_3) for adult humans at 2 to 5 milligrams; dangerous dose, 0.13 gram; for a horse the dangerous dose is 1.9 grams; for a cow 0.65 gram; and for a dog 32 to 64 milligrams. He says that poisoning of cattle can not occur through eating grass that has taken up arsenic from the soil through the roots.

Swain and Harkins (24) also think that plants do not absorb enough arsenic from the soil to poison animals. However, Formad's (11) investigations in the smelter region of the Northwest have shown arsenic deposited on pasture grasses to be a great menace to animals. He found arsenic in the grass and hay in amounts varying from 0.02 to 0.1 milligram of arsenious acid per gram of dry sample. Cattle, horses, and sheep feeding upon such grass and hay died by the thousands, evidencing all the symptoms of arsenic poisoning as developed in experimental treatments, and there seemed no doubt that arsenic was the cause of death. Harkins and Swain (15) analyzed the animals fed on the grass and hay containing arsenic and found it in all parts of the animals tissues.

Seddon & Ramsay (23) made several tests of feeding sheep and found the following fatal doses: sodium arsenite 14 grains, Paris green 17 grains, arsenic acid 16 grains. Lead arsenate was found to be definitely less toxic than the above compounds. A sheep given 60 grains died on the twelvth day while another given 120 grains lived.

In 1926 Janisch estimated the relative toxicity of certain stomach-poison insecticides in the following manner: He measured the area of each piece of cabbage leaf on cross-section paper, dusted the leaf by shaking it with the poison in a flask, weighed it again, and placed it with a single cabbage worm which fed on it until death. He measured the remaining leaf area and calculated the dose on the assumption that the dust was distributed evenly on the leaf surface.

Campbell & Filmer (8) improved on this method in devising a method which they term the sandwich method. In this method a uniform deposit of the insecticide in the form of a dust is placed on thin circular pieces of mulberry leaf.

A sandwich was then prepared from each disk in the following manner: A poisoned leaf disk was transferred by forceps to a clean glass plate. The smooth surface of a similar unpoisoned disk was coated by a camel's hair brush with a film of corn starch paste, made fresh every day. With forceps the disk was grasped by a vein on the other surface and carefully lowered upon the poisoned disk. The two disks were then pressed together by a cork thus completing the sandwich. An edge of each sandwich was inserted into a split half cork stopper so that it would be held firm in a position suitable for a silkworm to feed on it. A sandwich in its cork was then placed in each Petri dish with a silkworm.

Individual dosages were varied at will by taking the sandwiches from the larvae after they had eaten 1/8 to 1/2 of them. The dose then could be computed by calculating the area consumed by the silkworm.

Richardson & Haas (19) devised a method to determine the toxicity of various substances in poison baits for grasshoppers. The administration of known doses of the toxic compounds to the grasshoppers was accomplished by allowing them to consume small quantities of the bait from a tared dish, the dish and the grasshopper being retained in a moist chamber during the feeding process to prevent loss of moisture, and thereby loss of weight from the bait.

The procedure was as follows: about 0.5 gm. of the bait was placed in a small watch glass, quickly weighed on a chainomatic balance, and removed to a moist chamber. For this purpose bacterial culture dishes of 150 mm. outside arsenate and sodium fluosilicate. Mulberry leaves were sprayed with acid lead arsenate and with sodium fluosilicate, both at 2 pounds to 50 gallons of water. After the leaves had dried, silkworms of the fourth instar were allowed to feed freely on them. Mulberry leaves were also dusted heavily with the same compounds and the fourth instar silkworms were allowed to eat as much as they could. The effects of the spray and dust were compared in parallel and the comparison was repeated once. The period in which there was a 50 per cent mortality of each group of larvae was determined. For example, in a group of 40 larvae the survival period of the 20th worm to die was recorded.

Campbell also studied the relative effect of the same compounds on mosquito larvae, Culex Pipiens L., by the method of Marcovitch. Ten mature larvae of Culex Pipiens L. were placed in 50 cc. of water in each of eight 100 cc. beakers. The beakers were set in a water bath at 27° C. Four quantities of acid lead arsenate and of sodium fluosilicate 0.025, 0.050, 0.075, and 0.100 gram were weighed out on glass slides and were added rapidly to the beakers and stirred. Each of the four quantities of the fluosilicate was completely soluble in 50 cc. of water. The time was recorded at which the 5th larva in each beaker failed to respond by wriggling to gentle prodding with the stirring rod. The mean period of 50 per cent mortality is independent of the quantity of arsenate used but is dependent on the quantity of fluosilicate. The arsenate, being practically insoluble, settled to the bottom of the beakers where even the least of the four quantities used was much larger than the maximum quantity that the larvae could consume. Since the arsenate was equally accessible to the larvae in each beaker, about the same mean dose must have been taken in each beaker, resulting in the same mean period of 50 per cent mortality. The fluosilicate, being completely soluble, affected the larvae in proportion to its concentration, as all soluble poisons do when larvae are immersed in their solutions. As a result the fluosilicate appears from 0.6 to 1.8 times as toxic as the arsenate depending on the quantities of the poisons compared.

Hackenyos & Lilly (16) made a series of toxicity studies by hypodermic injection of the Larvae of Celerio Lineata. Campbell also used a similar method. The procedure of the first mentioned authors will serve to describe both methods in general. A B-D tuberculin hypodermic syringe graduated to 0.01 cc. and equipped with a 27 guage 3/4 inch platinum needle was used to administer the injections. A puncture was made in the body wall, slightly to one side of the mid-dorsal line on the second thoracic segment so as not to injure the heart mechanically. A sharp dissecting needle was used for this purpose and the opening was made just large enough for a small drop of blood to ooze out. The hypodermic needle was then inserted at the puncture and its point extended posteriorly into the larva for 1/2 inch just beneath the body wall. 0.05 cc. was the volume selected for all injections.

McIndoo & Cook (17) derived a method to estimate the minimum dosage of lead arsenate required to kill silkworms. By means of a needle-pointed pipette, an acid lead arsenate sample was dropped upon fresh mulberry leaves. Upon evaporation of the water from these drops, the portions of leaves bearing the white spots were fed to large hungry silkworms in the last instar. One drop would occasionally kill a large worm but more often two drops were fatal. In almost every case three drops proved fatal within 24 hours. Therefore for these larvae three drops may be regarded as a minimum fatal dosage of acid lead arsenate. An analysis of 100 drops (4 sets) from the same pipette gave 0.0091 milligram of metallic arsenic as an average per drop, making 0.0273 milligram of arsenic a minimum fatal dosage for fully grown silkworms.

McIndoo & Cook (17) also used a method in which the killed insects were analyzed by the Gutzeit method. In these experiments both sides of several mulberry leaves were heavily sprayed with acid lead arsenate. After having been dried by an electric fan, the leaves were fed to 50 large hungry silkworms. When the silkworms had ceased eating, they were removed to clean cages where the feces, contaminated as little as possible, were collected and subsequently analyzed. The next morning a sample of 34 dead and dying silkworms was thoroughly washed for five minutes in running tap water, then, one worm at a time, in six different washes, the first four consisting of hydrochloric acid (2 per cent) and distilled water and the last two of distilled water alone. A pencil brush was used for scrubbing them. Analysis of the sixth wash showed the presence of no arsenic. These experiments were repeated five times. To determine how much of the arsenic had passed through the intestinal walls, the alimentary canals of three sets were removed by careful dissection.

The results of the analyses of these samples were as follows: 84 entire silkworms yielded 2.66 milligrams of arsenic oxid, being 54 per cent water-soluble; 72 silkworms with alimentary canals removed yielded 0.89 milligram of arsenic oxid, being 36.7 per cent water-soluble; the alimentary canals of these 72 silkworms yielded 1.02 milligrams of arsenic oxid, being 55.9 per cent water-soluble; and the 2.18 grams of dried feces from these 72 silkworms yielded 0.45 milligram of arsenic oxid. According to the figures obtained from these 72 silkworms, 37.6 per cent of the total arsenic eaten had passed through the walls of the alimentary canals, 43.4 per cent of it was retained inside these canals, and 19 per cent of it was voided with the feces. Reaction (pH) of water extract from the larvae was neutral (7); from the alimentary canals, slightly alkaline (7.1); from the larvae with the alimentary canals removed, slightly acid (6.2); and from the feces, acid.

The foregoing experiments were repeated on a larger scale by feeding 13 arsenicals sprayed on leaves to caterpillars of the catalpa-sphinx moth (Ceratomia catalpae Boisd.). The results obtained indicate the following: (a) As a general rule, the higher the percentages of water-soluble arsenic in the larvae and feces, the higher the rates of toxicity of those arsenicals; (b) the percentage of water-soluble arsenic in the arsenical ingested usually has little to do with the rate of toxicity; (c) the amount of arsenic found per caterpillar is fairly constant for all the arsenicals used; (d) the higher the ratio of total arsenic (per 100 grams of larval material or feces) found in the larvae to that found in the feces, the higher the rate of toxicity; (e) the reaction (pH) of water extracts from the larvae fed various arsenicals seems to bear no relation to the rate of toxicity.

Haseman (14) in work on the apple worm attempted feeding experiments with apples treated with lead arsenate. After the solution of lead arsenate to be used was thoroughly shaken to distribute the arsenate of lead evenly, one, or in some cases two drops of the solution were dropped into a small cavity cut in a bit of apple. After slightly drying the treated bit of apple was then turned upside down over the worm in a small glass jar. In the majority of cases the worms at once set to work eating out the treated apple pulp, often leaving only a thin surface layer that usually dried so as to be unpalatable. In this way those worms which fed properly consumed the bulk of the treated pulp. By watching the worms, however, it was noted that some of the larger ones, that were not kept without food for a day or so just before making the tests, would cut off and discard bits of the surface treated pulp thus failing to consume the full dosage. The smaller hungry worms, however, usually fed properly. This tendency on the part of some worms to discard bits of the treated pulp made it difficult to determine just what part if not all of the dosage was consumed in such cases.

Campbell & Lukens (9) by means of a radioactive indicator method determined the rate of evacuation of a known dose of acid lead arsenate and of a known dose of basic lead arsenate.

A measured volume of a solution of thorium B in dilute acetic acid was mixed in a small glass cell with a measured volume of a solution of lead acetate. An aliquot of the mixture was withdrawn to serve as the standard and was placed in a watch glass. A radioactive acid lead arsenate was precipitated in the remaining solution by the addition of a solution of potassium dihydrogen arsenate. By first adding an excess of ammonia to the solution of potassium dihydrogen arsenate, a radioactive basic lead arsenate could be precipitated. In either case the precipitate was washed repeatedly by decantation until the pH of the supernatant liquid reached that of distilled water. The precipitate with a little water was then sucked into a capillary pipette and was immediately forced out upon a mulberry leaf disk (7/8 inch diam.) near its periphery and in a region free from the larger veinings. The resulting drop on the leaf disk was usually about 3 mm. in diameter. The leaf disk bearing the drop was placed under an electroscope to determine the radioactivity of the precipitate. From this measurement and the measurement of the standard, the quantity of lead in the precipitate was calculated. By multiplying the quantity of lead by a factor for acid lead arsenate or basic lead arsenate, the dose of either compound on the leaf disk was calculated.

OBJECT OF WORK

In considering colloidal arsenious sulphide in its application as an insecticide several things had to be considered. One of the most important phases has to do with the physical characteristics of colloidal arsenic. That is its stability, coverage or spreading ability, miscibility with various compounds especially alkali water, toxic effect on plants, adhesiveness. As many of these questions will be attempted to be answered as possible but in the limited time available not a very comprehensive study of these problems can be effected. No attempt was made to estimate the cost of this material.

The main portion of this work is concerned with establishing the effectiveness of colloidal arsenic or in other words its toxic value. As the amount of arsenic an insect eats at any one time is a rather difficult quantity to measure it was thought a more comprehensive study could be made by determining the amount of arsenic remaining in the food tube after death. By comparing the results obtained with colloidal arsenic to results obtained for standard poisons a measure of its toxic value should be obtained. This figure would not necessarily represent the minimum lethal dose due to absorption into the body tissues but it should be a figure by which the speed of toxic action could be determined. Also the amounts of different poisons found in the food tube will represent a measure of the amounts necessary to kill and thus be a comparison of their effectiveness.

The reason the food tube was used and not the entire grasshopper was that the hopper might pick up some arsenic on the outside of its body in crawling over the poisoned food which would introduce a great deal of error into the results.

Some attempt was also made to determine the effectiveness of colloidal arsenic as a contact insecticide.

TIME & PLACE OF WORK

All experiments were conducted in the research laboratory of the pharmacy department of South Dakota State College.

The grasshopper eggs from which the laboratory specimens were raised were dug during the last part of September 1932 by Gerald Spawn of the entomology department. The eggs were placed in cold storage until the 28th of Dec. 1932 when they were removed to 34 quart mason jars. The majority of the eggs hatched on the 14th of Jan. 1933. There was an average of 15 hoppers in a jar or over 500 grasshoppers. The adult stage was not reached by the hoppers until about the 1st week in April. A few hoppers still remained in the 6th or last instar before the adult on the 5th of May.

MATERIALS

The grasshoppers used in these experiments were all laboratory raised. The younger hoppers used in experiments were in about the 3rd instar and weighed from .1 to .15 gm. or an average weight of .125 gm. The older hoppers used ranged from .27 gm. to .77 gm. with an average weight of .426. The hoppers were raised from eggs furnished by the Entomology department of State College. We are indebted to H. C. Severin, George Gilbertson, and Gerald Spawn of the Entomology department for aid and advice in raising the hoppers.

All hoppers were kept in quart mason jars with a layer of sand in the bottom, the top being closed with a piece of gauze held in place with a rubber band. The food consisted entirely of fresh lettuce. The moisture in the jars was regulated by the appearance of the sand.

The poisons used in these experiments were colloidal arsenious sulphide (As_2S_3) containing 0.006 gm. of arsenic per cc., Paris Green $(CuOAs_2O_3)_3$. $Cu(C_2H_3O_2)_2$, Arsenic trioxide not more than 50 per cent and not more than 3½ per cent of water soluble arsenic and sodium arsenite NaH_2AsO_3.

The poisoned bait consisted of wheat bran, molasses, a poisonous compound, and water.

The bran was a coarse, flaky product such as was sold throughout South Dakota for poison bran mash. The molasses was a commercial grade put up in tin cans for table use. Distilled water was used in all the bait preparations.

The digestion flasks used in the Gutzeit test for arsenic were 250 cc. Erlenmeyer flasks. All flasks, before being used, were heated with a concentrated solution of sodium hydroxide, then washed three times with distilled water, then washed with a 5 per cent HCl solution followed by two more washes of distilled water. The flasks were fitted with one hole rubber stoppers having a short glass rod pushed through the stopper, the rod being slightly contracted just above the top of the stopper to prevent the sensitized paper from slipping through. Before being used the stoppers and rods were boiled for 30 minutes in a strong solution of sodium bicarbonate and then given the same treatment as above for the flasks.

The sensitized paper was made by cutting standard strips 3/16 in. by 2½ in. from ashless filter paper and treating with a 5 per cent mercuric bromide paper.

The bottom of each glass rod holding a sensitized paper was loosely stoppered with a piece of cotton which had been soaked in lead acetate T. S. and allowed to dry.

The hoppers were digested with Folin's digestion mixture made up according to the following formula. Phosphoric acid (85%) As free 600 cc., Sulphuric Acid (94%) As free 300 cc., Copper sulphate - reagent quality As free 80 gm. To facilitate digestion a few drops of hydrogen peroxide were added to the flask before heating. In most cases the hoppers were digested until the solutions were clear and showed no brown color.

The poisoned lettuce used was prepared by dipping in the solution of colloidal arsenic, and in the case of paris green by dipping in a solution prepared according to the same strength used in the field or at the rate of 2 lb. paris green to 50 gallons of water or 4.8 gm. in 1,000 cc.

The zinc used in the Gutzeit test was As free.

The standards used for comparison in the Gutzeit test were prepared from a solution of arsenic trioxide which was carefully standardized. All standards were checked by titration as well as by the Gutzeit method.

The procedure for preparation of standard bands is given by Autenreith & Warren: Dissolve 1 gram of resublimed arsenious oxide in a little arsenic - free sodium hydroxide, acidify with sulphuric acid, and make up to a liter with recently boiled water. Dilute 10 cc. of this solution (I) to a liter with freshly boiled water which gives solution (II) containing 0.01 mg. of arsenious oxide per cc. Using definite volumes of solution II, measured from a burette, prepare a series of color-bands, taking a fresh charge of zinc and acid for each portion. The color ranges from lemon-yellow through orange yellow to reddish brown.

METHODS

As the object of the experiment was to determine the amount of arsenic in the food tube no attempt was made to feed the grasshoppers any specific or known amount of poison.

The poison bran mixture used was made up according to the following formula: Bran 25 grams, poisonous ingredient 1 gram, molasses 6 grams, and water 35 cc. In the case of colloidal arsenic since the poison was in a liquid form the formula used was colloidal arsenic solution 35 cc. Bran 25 grams, molasses 6 grams. The baits were made up fresh for each group of trials.

The procedure was as follows: a grasshopper was weighed and placed in a separate quart mason jar. It was left without food until the following day. The poisoned food was then placed in the jar. In the case of poisoned lettuce the hoppers fed eagerly but in the case of poison bran they did not show the same inclination to feed. The poisoned food was left in the jar overnight and the next day was replaced by fresh lettuce. In the case of poison bran the hopper was usually removed to a fresh clean jar. The criterion of death was the inability of the insect to move when the hind tarsus was pinched.

When a hopper was found to be dead it was removed from the jar and the food tube was carefully dissected out and placed in an Erlenmeyer flask. In some cases the food tube was divided into two parts making a cut just behind the gastric caeca. In nearly every case the crop and oesophogus was found to contain more poison than the remainder of the food tube.

The hopper was then digested with Folin's digestion mixture, digestion being continued until the solution was clear and colorless. 5cc. of Folin's mixture was used for this purpose in every case. 20 cc. of water were added to the flask. About 5 small zinc shot were added and the flask immediately stoppered with the rubber stopper containing a glass rod and strip of sensitized paper. The edge of the stopper as well as the portion around the glass rod was sealed with paraffin.

After leaving the flask overnight the sensitized strip was removed and compared to known standard and the amount of arsenic in micro-milligrams could be easily calculated by direct comparison.

Experimental grasshoppers were accompanied by controls which fed bran bait without poison during the same period as those which were poisoned and then placed on fresh lettuce. This treatment had no visible effect on them.

About 80 per cent of the control insects survived more than 120 hours. A number of insects died when the jars became too damp, probably from fungus disease. Individuals in these groups were discarded unless they survived over several weeks time when placed in new jars with fresh lettuce.

As the number of individuals dying in each survival period from causes other than poisoning was approximately equal and not excessively large, the mortality in the controls has been disregarded in calculating the dosages for the several toxic compounds.

EXPERIMENTS & RESULTS

The results of experiments with colloidal arsenic, paris green, and sodium arsenite using two kinds of food, poison bran and poisoned lettuce are given in tables.

Early experiments with young hoppers in the 2nd and 3rd instar were conducted entirely with colloidal arsenic. The other two poisons, paris green and sodium arsenite were not used until the hoppers were adult or nearly adult. Every group of ten hoppers tested by the Gutzeit method was accompanied by two control hoppers which had not been fed poison. Under proper conditions not more than a slight tinge of yellow showed upon the control flasks. This color did not extend up the strip over 1/32 of an inch. In case more than this amount of arsenic showed up the entire series had to be discarded. Much time was wasted in the earlier experiments due to a lack of proper procedure in removing the arsenic from the Erlenmeyer flasks. The results from not more than 1/4th of the insects tested were considered sufficiently reliable to be used. Although only a small number of individuals were considered in making up the toxicity table the figures shown are more significant than might first appear due to the fact that any series which the blank test did not check on were discarded. In nearly all cases when the blank tests showed presence of arsenic in the flask the other tests in the series showed that they were unsatisfactory by the presence of unreasonable amounts of arsenic showing up on the strips. In an unsatisfactory series at least two or more of the strips would be covered with a deep brown color. In a satisfactory series all strips would show a rather uniform color of varying heights but none entirely covering the strip. The higher the color on the strip the deeper the color would be.

Experiments with young hoppers indicated that death occured more quickly to the greatest number when colloidal arsenic was fed on poisoned lettuce than when fed in poison bran mixture. The poison bran mixture did not seem to be particularly attractive to the young hoppers. The individuals unfortunately were only observed at 24 hour intervals. Dead hoppers which had been fed poisoned bran were found to contain an average of .042 mg. of arsenic per gm. of body weight. Richardson places the dosages in milligrams per gram of body weight into 4 groups according to survival time periods. In group three he places those dosages which required the longest time to kill and in group four he places those dosages which permitted recovery. He then estimates that there should be a point somewhere between the two groups that would represent the true median lethal dose (M. L. D.), i. e. the dose at which half the population is killed and half survives. As the amount of arsenic in individuals which recovered was not determined in this work the figures we obtained will be more nearly comparible to the minimum lethal dose (M. L. D.) as it is used in pharmocological literature or in other words the least amount to kill. Although the figures given in this work are not an exact measure of the least amount to kill due to absorption of arsenic into the body tissues they can be taken to represent the least amount found in the food tube which will cause death. In other words the figures M. L. D. as found throughout this paper represent the minimum lethal dose which must be found in the food tube to cause death of a grasshopper.

In the case of the young hoppers then in which we found .042 mgm./gm. of arsenic in the food tube we will also consider the M. L. D. to be .042 mgm./gm. The young hoppers which were fed on poisoned lettuce were found to contain slightly more arsenic or an average of .052 mgm./gm.

Older hoppers some of which were in the 6th instar and some of which were adult seemed even less inclined to eat the poison bran which was treated with colloidal arsenic than the young hoppers. The older hoppers given this food had longer survival periods than those which were fed on poison bait mixtures of sodium arsenite and of paris green. Those fed the paris green poison bait mixture had shorter survival periods than those fed the sodium arsenite poison bait mixture although the two were approximately equal (Graph No. III).

The most successful material that was used from the standpoint of poisoning was lettuce which had been treated with colloidal arsenic. A majority of the deaths occured within 3 hours and a very few lived over 24 hours. The largest amount of arsenic was found in this group as might be expected or an average of .151 mgm./gm. However taking an average with those which died in 24 hours the M. L. D. is found to be .115 mgm./gm, a figure which agrees very well with those found for sodium arsenite and paris green. (Graph No. II)

Experiments conducted on paris green indicate that it was quite effective in poison bait material but very ineffective when applied to lettuce. This probably is due to the fact that the paris green is repellant to the grasshoppers and they eat around it. The grasshoppers which were treated in this manner had very long survival periods. In fact it is very doubtful if the paris green had much to do with their death. The amount of arsenic found in these hoppers was so small that the results were disregarded as being unreliable. However a record was kept of the survival time and is presented in the tables. The average M. L. D. for the poison bran paris green mixture was .085 mgm./gm. which was higher than for sodium arsenite and colloidal arsenic which might be expected since it was quicker in action in this case.

Tests were conducted with sodium arsenite using only poison bran as a carrier since there were not sufficient hoppers remaining alive at this period to conduct experiments with poisoned lettuce. The average M. L. D. was found to be .059 mgm./gm. This figure lying between the results found for colloidal arsenic and for paris green.

Some experiments were conducted to determine if paris green & colloidal arsenic had any effect as contact poisons. In the first set of experiments the antennae of 4 grasshoppers were painted with colloidal arsenic and the antennae of 4 grasshoppers were painted with paris green mixed in water. In each case two out of the four hoppers died in about 48 hours. Upon examination for arsenic the hoppers treated with paris green were found to contain about 10 micromilligrams of arsenic and those treated with colloidal arsenic about 2 to 3 micromilligrams. As a record was not kept of the weight of these hoppers these figures are not significant.

These experiments were repeated using 2 hoppers for paris green and 2 hoppers for colloidal arsenic. In this case however the individuals were painted on the back so they could not reach the poison with this mouth parts. A camel's hair brush was used for the painting in all cases.

The first symptoms exerted by the hoppers in both cases was a profound irritation and a violent stretching of the abdomen and a seemingly evidence of diarrhea. This continued for some hours but at the end of 24 hours the individuals had returned to normal even though the paris green could be seen to be dried on the back of the grasshopper. At the end of 48 hours the same hoppers were again painted and went through the same procedure as before. At the end of 48 hours or a total of 96 hours all individuals were living and were considered as recovered. The conclusion derived from this experiment was that colloidal arsenic and paris green have no effect as a contact insecticide and those individuals which died when their antennae was painted must have obtained a poisonous dose by drawing their antennae through their mouth parts.

The preparation of a stable solution of colloidal arsenic took considerable time and experimentation.

It was first thought that a stable solution could be prepared by adding a solution of colloidal ferric hydroxide to one of colloidal arsenious sulphide. As colloidal iron hydroxide is a positively charged colloidal and arsenious sulphide is a negatively charged colloid the charges tend to neutralize one another and at the iso-electric point or neutral point there is complete precipitation. This only at both ends of a series, either a great excess of iron hydroxide or a great excess of arsenious sulphide do we have any degree of stability. In the case of the excess of iron hydroxide it was possible to add only such a small amount of arsenious sulphide as to make it impracticable to use. In the case of a large excess of arsenious sulphide solution the small amount of iron hydroxide did not materially add to the stability. This method was then abandoned.

Solutions of colloidal arsenious sulphide were prepared in the following manner: arsenic trioxide was strongly boiled with water (1 gm. per 100 cc. were used). This solution was cooled and then filtered. H_2S was then passed into this solution, first passing the H_2S through water and then to the arsenic solution. The excess H_2S was removed by bubbling through hydrogen generated with zinc and HCl. These solutions thus prepared are not very stable.

The addition of a small amount of gelatin (60 cc. of 1% gelatin to 1000 cc. of solution) produced a stable solution which did not settle out in 2 months period and gave only a slight precipitate at the end of 3 months time. Considerable difficulty was experienced at first by the formation of growths in the solutions. The addition of thymol (0.2 gm. per 1000 cc.) prevented this.

EXPERIMENTS ON THE EFFECT OF HARD WATERS ON DILUTING

COLLOIDAL ARSENIOUS SULPHIDE SOLS.

Solutions of colloidal arsenious sulphide containing 0.006 gm. of arsenic per cc. and made stable with gelatin were found to stand up very well when mixed with hard water.

Colloidal arsenic when diluted 1-5 with distilled water containing 5 per cent mg. SO_4 gave no precipitate at the end of three weeks and only a slight precipitate at the end of six weeks.

Colloidal arsenic was diluted 1-5 with Brookings city tap water of the analysis.#

Cl -	6.28	p. p. m. (parts per million)
Alkalinity (As$CaCO_3$)	287.2	p. p. m.
SiO_2	28.5	p. p. m.
Fe and Al oxides (R_2O_3)	13.0	p. p. m.
Ca	150.7	p. p. m.
Mg.	49.7	p. p. m.
SO_4	302.0	p. p. m.
Na	24.75	p. p. m.

This acted the same as the above and gave no precipitate at the end of 3 weeks and only a slight precipitate at the end of 6 weeks.

Colloidal arsenic was diluted 50-50 with very hard water from Hot Springs, S. Dak. of the analysis.#

Cl	20 p. p. m.
Alkalinity (As $CaCO_3$)	56.0 p. p. m.
SiO_2	20.5 p. p. m.
Fe and Al (R_2O_3)	56.5 p. p. m.
Ca	348.5 p. p. m.
Mg	459.7 p. p. m.
SO_4	5,058.5 p. p. m.
Na	1,169.5 p. p. m.
Total solids	7,189.3 p. p. m.

Although this was an extremely hard water the solution did not settle out over 2 months time.

The conclusion derived from this is that if it is to be used immediately colloidal arsenic can be mixed with most any hard water. In all probability such solutions would be stable about a month.

We are indebted to Alvin Moxin of the State College Experiment Station for the above analysis.

TABLES OF RESULTS

The following tables show the results accomplished with three poisons: colloidal arsenic, paris green, and sodium arsenite. The tables are grouped according to the time of kill and the kind of carrier used for poison. The letters M. L. D. used throughout these tables signify minimum lethal dose.

C O L L O I D A L   A R S E N I C

YOUNG HOPPERS (2nd-3rd. Instar) Average Weight .125 Gm.

Poison Bran Killed in 24 hours.

No.	Dose in Micromilligrams	Dose in Mgm. per Gm. of Body Weight
1	5	.04
2	8	.064

Average M. L. D. For Group = .052

Poison Bran Killed in 48 hours.

1	5	.04
2	3	.024
3	3	.024
4	5	.04

Average M. L. D. for Group = .032

Poison Lettuce Killed in 24 hours.

1	8	.064
2	6	.048

No.	Dose in Micromilligrams	Dose in Mgm. per Gm. of Body Weight
3	8	.064
4	10	.080

Average M. L. D. For Group = .064

Poisoned Lettuce Killed in 72 hours.

1	5	.04
2	5	.04

Average M. L. D. For Group = .04

OLDER HOPPERS (6th Instar to Adult)

Weight of Hopper	Dose in Micromilligrams	Dose in Mgm. Per Gm. of Body Weight
.3560	45	.127
.2711	25	.092
.2740	50	.182
.3550	60	.168
.3784	70	.185

Average M. L. D. For Group = .151

Poisoned Lettuce Killed in 24 hours.

.2113	20	.095
.2758	20	.072

Average M. L. D. For Group = .084

Poison Bran Killed in 24 hours.

.4762	30	.063

M. L. D. = .063

Poison Bran Killed in 60 hours.

Weight of Hopper	Dose in Micromilligrams	Dose in Mgm. Per Gm of Body Weight
.6120	9	.0146

M. L. D. = .0146

Poison Bran Killed in 96 hours.

.7790	65	.083
.4981	50	.100

Average M. L. D. = .091

PARIS GREEN

Poison Bran Killed in 24 hours.

.6030	80	.132

M. L. D. = .132

Poison Bran Killed in 48 hours.

.3311	10	.030
.3141	5	.016
.4493	14	.031
.2860	23	.080

Average M. L. D. = .039

Poisoned Lettuce Killed in 144 hours.

.6462	14	.022

M. L. D. = .022 #

This figure not used in summary tables.

SEVERAL HOPPERS WERE KILLED WITH LETTUCE POISONED WITH PARIS GREEN BUT AMOUNT OF ARSENIC WAS NOT DETERMINED.

Weight of Hopper	Survival Time in Hours.
.4496	120
.5412	120
.4762	144
.2742	144
.3972	144
.4482	148
.3162	148

SODIUM ARSENITE

Poison Bran Killed in 24 hours.

Weight of Hopper	Dose in Micromilligrams	Dose in Mgm. Per Gm. of Body Weight
.4138	56	.123

M. L. D. = .123

Poison Bran Killed in 48 hours.

.4233	12	.028

M. L. D. = .028

Poison Bran Killed in 60 hours.

.3162	9	.028

M. L. D. = .028

COMPARISON OF DECREASE IN DOSE OF ARSENIC OVER MORE THAN 24 HOURS.

Kind of Food	Poison		Average M. L. D. 24 hrs. or less	M. L. D. Over 24 hrs.	% Decrease
Lettuce	Colloidal	Young	.064		
		Old	.151	.062	45.7%
		Both	.107		
Poison Bran	Colloidal	Young	.052	.032	38.4%
		Old	.063	.053	15.8%
		Both	.057	.042	26.3%
	Paris Green		.132	.039	70.4%
	Sodium Arsenite		.123	.028	77.2%
Both Kinds of Food	Colloidal		.081	.052	35.8%

COMPARISON OF TIME FOR 3rd HOPPER OUT OF 5 TO DIE

(Graph No. III)

Bran -	Sodium Arsenite -	60 hours.
Bran -	Paris Green -	48 hours.
Bran -	Colloidal Arsenic -	96 hours.
Lettuce -	Colloidal Arsenic -	3 hours.
Lettuce -	Paris Green -	144 hours.

COMPARISON OF DECREASE IN DOSE OF ARSENIC OVER MORE

THAN 24 HOURS.

Kind of Food	Poison	Average M. L. D. 24 hrs. or less		M. L. D. Over 24 hrs.	% Decrease
Lettuce	Colloidal	Young	.064		
		- Old	.151	.062	45.7%
		Both	.107		
Poison Bran	Colloidal	Young	.052	.032	38.4%
		- Old	.063	.053	15.8%
		Both	.057	.042	26.3%
	Paris Green		.132	.039	70.4%
	Sodium Arsenite		.123	.028	77.2%
Both Kinds of Food	Colloidal		.081	.052	35.8%

COMPARISON OF TIME FOR 3rd HOPPER OUT OF 5 TO DIE

(Graph No. III)

Bran -	Sodium Arsenite -	60 hours.
Bran -	Paris Green -	48 hours.
Bran -	Colloidal Arsenic -	96 hours.
Lettuce -	Colloidal Arsenic -	3 hours.
Lettuce -	Paris Green -	144 hours.

COMPARISON OF TIME OF KILL TO DOSE (GRAPH NO. I)

Kind of Poison	Kind of Food	Age of Hoppers	Dose	Time
Colloidal Arsenic	Bran	2-3 instar	.052 .032	24 hours.
Colloidal Arsenic	Lettuce	2-3 instar	.064 .04	24 hours.
Colloidal Arsenic	Bran	6th instar to adult	.063 .0146	24 hours. 60 hours.
Colloidal Arsenic	Lettuce	"	.151 .084	3 hours. 24 hours.
Paris Green	Bran	"	.132 .039	24 hours. 48 hours
Sodium Arsenite	Bran	"	.123 .028 .028	24 hours. 48 hours. 60 hours.

COMPARISON OF MINIMUM LETHAL DOSE AT END OF 24 HOURS WITH RICHARDSON'S MEDIAN LETHAL DOSE

(GRAPH NO. II)

MY RESULTS

Poison	Dose
Colloidal Arsenic	.115
Sodium Arsenite	.123
Paris Green	.132

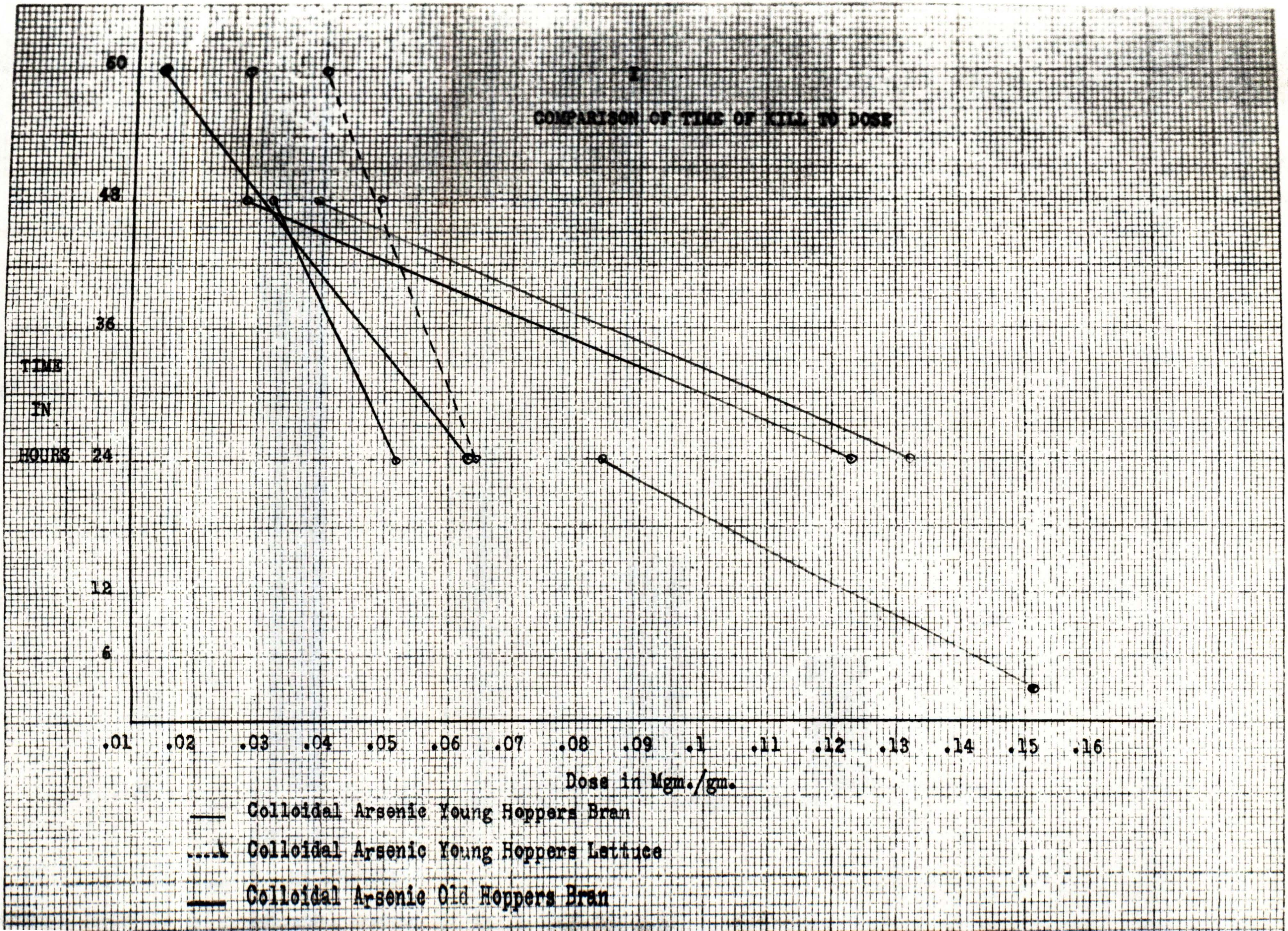
Richardson's Results

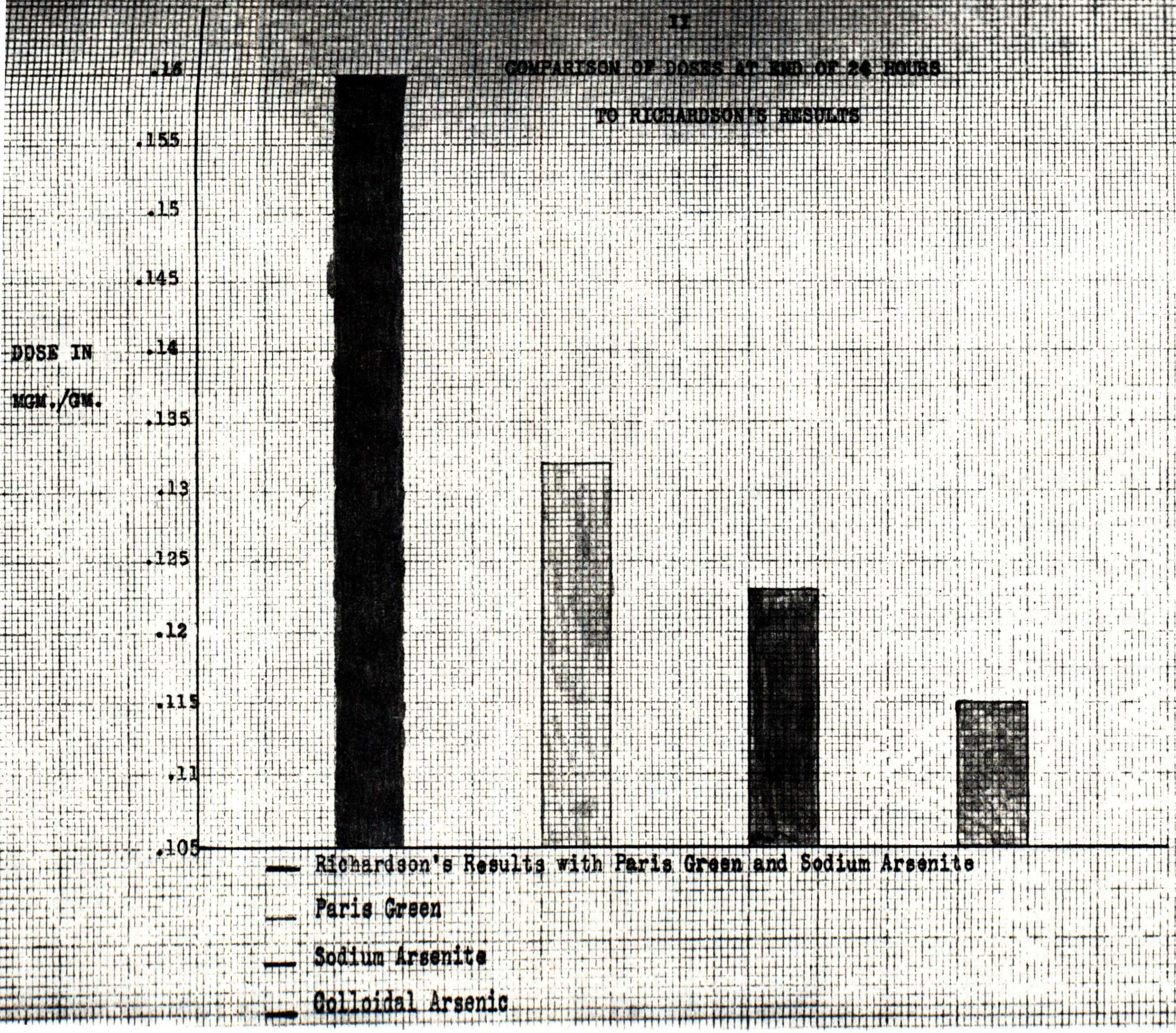
Poison	Dose
Sodium Arsenite	.16
Paris Green	.16

COMPARISON OF SPEED OF TOXIC ACTION $\left(\frac{1000}{\text{Time in Hours}}\right)$

TO DOSE (GRAPH NO. IV)

Kind of Poison	Kind of Food	Age of Hoppers	Dose	Speed of Toxic Action
Colloidal Arsenic	Bran	2-3 instar	.052 .032	4.2 2.1
Colloidal Arsenic	Lettuce	2-3 instar	.064 .04	4.2 1.4
Colloidal Arsenic	Bran	6th instar to adult	.063 .0146	4.2 1.7
Colloidal Arsenic	Lettuce	"	.151 .084	33.3 4.2
Paris Green	Bran	"	.132 .039	4.2 2.1
Sodium Arsenite	Bran	"	.123 .028 .028	4.2 2.1 1.7


I
COMPARISON OF TIME OF KILL TO DOSE
TIME IN HOURS
60
48
36
24
12
6
.01
.02
.03
.04
.05
.06
.07
.08
.09
.1
.11
.12
.13
.14
.15
.16
Dose in Mgm./gm.
Colloidal Arsenic Young Hoppers Bran
Colloidal Arsenic Young Hoppers Lettuce
Colloidal Arsenic Old Hoppers Bran



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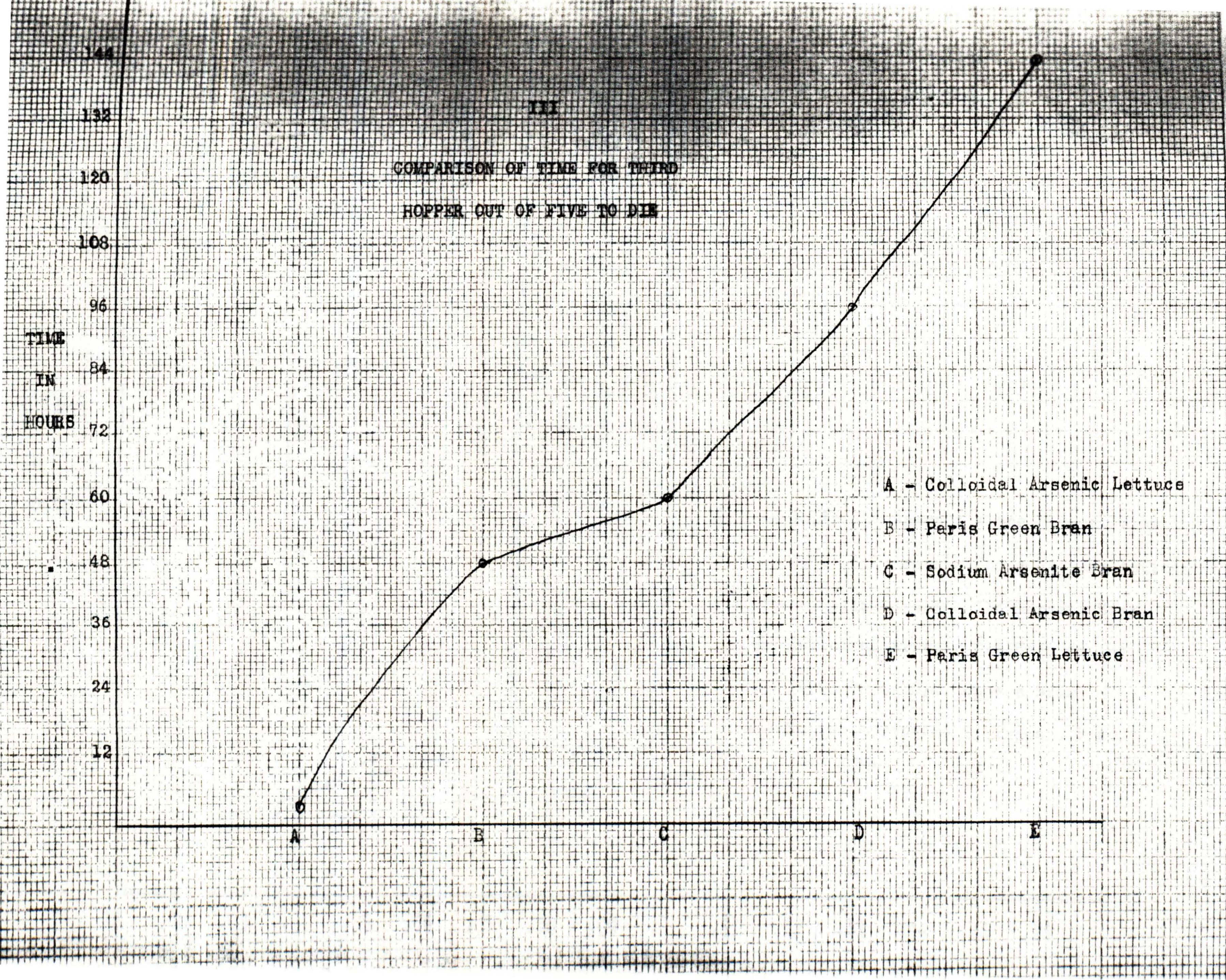

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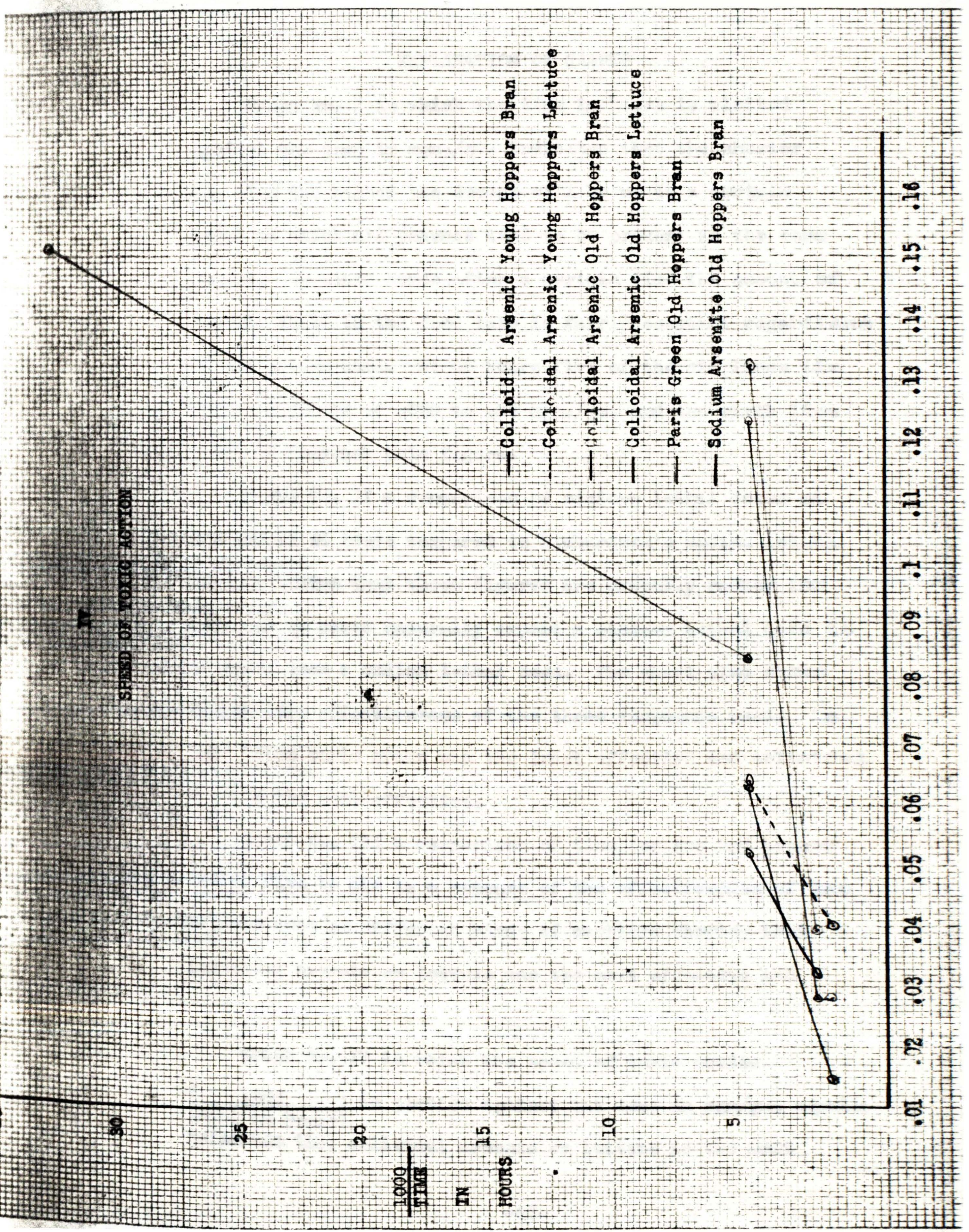

EXPLANATION OF GRAPHS

Graph No. I in which the time in hours is plotted against the dose in mgm./gm. indicates that the shorter the time the higher the dosage will be up to a certain point at which all poisons tend to be present in the same amount. In other words after a certain time the amount of arsenic found in the food tube is fairly constant regardless of the poison used. At 48 hours five of the six lines lie within a dosage of .028 to .04 mgm./gm. and the sixth line tends to approach this figure.

Graph No. II a comparison of minimum lethal doses at the end of 24 hours compared to Richardson's median lethal dose indicates that these figures are probably rather low although we would naturally expect a minimum lethal dose to be lower than a median lethal dose. The chart also shows comparative effectiveness of the three compounds used. On this basis paris green was the least effective and colloidal arsenic the most effective.

Graph No. III a comparison of time for the 3rd hopper out of five to die is a measure of the effectiveness of the poisons used on different kinds of carriers showing that colloidal arsenic on lettuce was the most effective and paris green on lettuce was the least effective.

Graph No. IV in which the speed of toxic action $\left(\frac{1000}{\text{Time in Hours}}\right)$ is plotted against the dose in mgm/gm. again indicates that colloidal arsenic on lettuce is the most effective material on the basis of speed of toxic action. It also indicates that the speed of toxic action of paris green and sodium arsenite are about the same and that the speed of toxic action of colloidal arsenic is greater than either of these two compounds.

PRELIMINARY EXPERIMENTS ON SPRAYING FOLIAGE

For the spraying experiments a concentrated colloidal arsenic solution was made up as follows: 25 gm. of As_2O_3 were boiled with 800 cc. of distilled water for 3 hours. Solution cooled and filtered. Solution measured and found to be 720 cc. Residue remaining on filter paper was weighed and found to be 2 gm. This would give an equivalent of .032 gm. of As_2O_3 per cc. of solution. H_2S was run into the solution for two minutes and a current of hydrogen was passed through the solution for five minutes. This solution was again filtered, 25 cc. of 8 per cent gelatin and .7 gm. of thymol were added.

In the first experiments 37.5 cc. of the above concentrated solution and 25 cc. of milk were diluted to 500 cc. This gave a solution whose strength was equivalent to 1 lb. of As_2O_3 to 50 gallons of water. This was applied to a plum tree and a rasberry bush about 2:00 P. M. on a bright sunshiny day. The edges of the leaves of the rasberry plant were burned in 24 hours. No effect showed up on the plum tree at the end of six days. It was thought that the reason the plum tree did not show burning was that the leaves had a very waxy surface and the solution did not stick to the leaves.

A second solution was made up containing an equivalent of 1/2 lb. of As_2O_3 to 50 gallons of water. 25 cc. of water glass were added to 475 cc. of this solution. The addition of water glass greatly increased the adhesiveness of the mixture. When applied to rasberry bushes and plum trees it caused severe burning in 24 hours.

A solution containing the equivalent of 1 ounce of As_2O_3 to 50 gallons of water was applied to box elder, currant bushes and rhubarb. Severe burning was present on the leaves of the box elder at the end of 4 days. The other two plants showed no injury.

It is evident from the above experiments that when colloidal arsenic is used in an effective concentration some material must be added to prevent burning. As yet no material has been found which will prevent burning and not precipitate the colloidal arsenic.

DISCUSSION OF RESULTS

Generalizations

McIndoo & Cook state that as a general rule the larger the average amount of arsenic found in the insects analyzed, the higher is the rate of toxicity of that arsenical. In their experiments the entire insect was analyzed. This was found to be true in this work, the quicker an arsenical killed the more arsenic was found in the food tubes. However in evaluating the killing power of an arsenical, the smaller the amount of arsenic found in the food tube the greater the killing power. This should be true because if an arsenical is present in the food tube in a smaller amount more of the arsenicals must have passed through the walls of the alimentary canal into the body tissues. In order to make this comparison insects had to be compared on the same time basis of kill.

The fact that all poison tends to approach a constant value after 48 hours can be explained by the supposition that a nearly constant amount of arsenic is fixed in the food tube of the grasshoppers after a certain length of time the remainder either passing through the walls into the body tissues or being voided with the feces.

The experiments with colloidal arsenic used upon poisoned lettuce may have a practical value in control of grasshoppers since they show that this material is more attractive than poisoned bait and also considerable more rapid in its killing power. In actual application the colloidal arsenic might be sprayed upon some natural food of the grasshopper which was not deemed worthy of cultivation.

COMPARISON TO OTHER RESULTS

No direct comparison can be made with any other results in view of the fact that we are comparing the amount of arsenic found in the food tube after death with the amount of poison used to produce death either by feeding or injecting known amounts. Also we are comparing amounts of arsenic with total amounts of poison.

However a rather interesting relationship can be shown by arranging the results of other authors in order of the amount of poison used and including my results with these. This table is given below:

Author	Insect	Poison	Dose in Mgm./gm.
Richardson & Haas	Potato Beetle	Lead Arsenate	0.3
Van Leeuwen	Japanese Beetle	" "	.035
Campbell & Filmer	4th instar Silkworm	" "	.09
Richardson & Thurber	Grasshopper	Arsenious Oxide	.11
My Results	"	Colloidal Arsenic	.115
" "	"	Sodium Arsenite	.123
" "	"	Paris Green	.132
Richardson & Haas	"	" "	.16
" "	"	Sodium Arsenite	.16

SUMMARY OF RESULTS

1. The average amount of arsenic found in the food tube of the grasshopper (Melanopus differentialis) at the end of 24 hours using two kinds of food was found to be .115 mgm./gm. of body weight for colloidal arsenic, .132 mgm./gm. for paris green, and .123 mgm./gm. for sodium arsenite.
2. The average amount of arsenic found in the food tube over all time periods using bran as a carrier was found to be .056 mgm./gm. of colloidal arsenic, .059 mgm./gm. of sodium arsenite, and .085 mgm./gm. of paris green.
3. The amount of arsenic found in the food tube for all poisons and all kinds of food used as a carrier was found to approach a constant value at the end of 48 hours and ranged from .028 mgm./gm. to .04 mgm./gm.
4. The most effective method of poisoning was found to be colloidal arsenic using lettuce as a carrier, deaths occurring within 3 hours. Paris green was found to be very ineffective when applied to lettuce.
5. Paris green and sodium arsenite had about the same rate of speed of toxic action in poison bran. Colloidal arsenic in poison bran was less effective than these compounds.
6. Colloidal arsenic and paris green were found to have no effect as contact insecticides.

7. Colloidal arsenic was found to be quite stable when diluted with hard waters.

8. Colloidal arsenic used at a concentration equivalent to 1/2 lb. of As_2O_3 to 50 gallons of water caused severe burning to plum trees and rasberry bushes.

9. No material has been found which may be added to colloidal arsenic solutions which will prevent burning and not precipitate the colloidal arsenic.

10. The following is a partial list of the unsolved problems in connection with this work:

    A. Concentration which must be used in the field to be effective on different types of plants and for different types of insects.

    B. Length of time which solution will be effective against insects on plants.

    C. Material which may be added to colloidal arsenic to prevent burning.

    D. Convenient and simple method of removing residue of colloidal arsenic.

    E. Effect of galvanized iron and other materials used in pipe lines on colloidal arsenic.

    F. Physiological effect of colloidal arsenic upon animals.

    G. Wetting and spreading ability of colloidal arsenic.

H. Weather conditions which are best for application of colloidal arsenic.

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I N D E X

I. Title

II. Introduction

A. Nature of problem, scope, bearing and importance.

B. Review of important literature.

C. Object of work.

D. Time and place of work.

III. Materials and Methods

A. Description of equipment and materials.

B. Explanation of method.

IV. Experiments and Results

A. Detailed description.

B. Description of results.

V. Discussion of Results

A. Generalizations.

B. Comparison to other results.

VI. Summary

VII. Bibliography